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Helium-Neon Laser Irradiation at Fluences of 1, 2, and 4 J/cm² Failed To Accelerate Wound Healing as Assessed by Both Wound Contracture Rate and Tensile Strength

John D.F. Allendorf, BA, Marc Bessler, MD,* James Huang, BA, Mark L. Kayton, MD, Dennis Laird, MD, Roman Nowygrod, MD, and Michael R. Treat, MD

Columbia University and Columbia-Presbyterian Medical Center, New York, New York, 10032

Background and Objective: **Reports in the literature indicate that low energy laser irradiation has a biostimulatory effect on wound healing; however, no mechanism of this effect has been elucidated.**
Study Design/Materials and Methods: **We attempted to establish a model from which to study the mechanism of biostimulation. The effects of low energy helium-neon irradiation on wound healing were observed in two rat models. In the first model, 1.5 cm diameter full thickness excisional skin defects were created in the dorsal midline of rats (n = 32). All animals were anesthetized and all eschars were debrided daily. Wound area was determined by caliper measurements for 2 weeks postoperatively. Rats that received a treatment of 1 J/cm² had two defects in the dorsal skin. One wound was treated and the second was used as its own control. These measurements were not blinded. Rats that received 2 J/cm², 4 J/cm², or anesthesia alone had one defect on the dorsal skin. Caliper measurements of these wounds were blinded. We were unable to demonstrate any difference in the rate of wound contracture in rats that received a daily dose of 1 J/cm², 2 J/cm², 4 J/cm², or anesthesia alone ($P > 0.8$ by student's t-test). In the second model, a single 2 cm longitudinal full thickness skin incision was created in the dorsal midline of each rat (n = 24). No difference was found between rats that received anesthesia alone and those treated daily with 2 J/cm² as assessed by tensile strength measurements on postoperative days 7 and 14 ($P > 0.8$ by student's t-test between groups at both time points). These determinations were blinded.**
Results: **Despite our intentions of studying the mechanism of low energy HeNe biostimulation, we were unable to demonstrate a beneficial effect.**
Conclusion: **In this study, helium-neon laser irradiation produced no measurable benefit on wound healing. Lasers Surg. Medicine 20:340–345, 1997.**

Key words: **excision; incision; rat; HeNe; helium-neon; laser; wound healing; low energy**

INTRODUCTION

Low energy helium-neon (HeNe) laser irradiation has been shown by several authors to ac

*Correspondence to: Marc Bessler, M.D., 177 Fort Washington Avenue, MHB-7SK, NY, NY 10032.

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celerate the wound healing process [1–11]. Work in Eastern Europe and Russia pioneered by Mester [7–9] demonstrated increased phagocytosis by lymphocytes, increased wound contraction rate, increased healing of burns, and increased collagen synthesis following low energy laser irradiation. Other authors have demonstrated increased colon bursting strengths, enhanced fibroblast production of collagen, and fibroblast differentiation into myofibroblasts in response to low energy HeNe exposure [2,12]. The basic mechanism of HeNe energy transduction into biological activity has not been elucidated; however, authors have hypothesized that the HeNe biostimulation phenomenon works via increases in procollagen mRNA production, increased crosslinking of existing collagen molecules, acceleration of epithelial repair, and growth of granulation tissue [6,7,11].

Clinically, low energy laser irradiation has been used to treat skin ulcers resistant to healing. In open trials involving hundreds of patients, Mester [9] demonstrated excellent results. However, a controlled study conducted by Lundeberg and Malm [17] involving 46 patients failed to demonstrate significant differences. In addition, smaller open clinical studies by Santoianni [18] and Brunner [19] were unable to substantiate Mester's success. Although Mester recommends the use of low-energy lasers in clinical settings, many laboratories are still unable to repeat his laboratory results.

Several other reports fail to substantiate the above laboratory results [1,3,4,13–15]. Among these, Braverman [1], Surinchak [4], and Hunter [13] reported no increased rate of wound contraction in an excisional model in direct conflict with Mester's findings. Thus laser efficacy for wound healing remains unestablished. We used both an excisional and an incisional model similar to those described by earlier reports in an attempt to study the mechanism of HeNe accelerated wound healing, to generate a dose response curve, and to help resolve the discrepancies among reports in the literature.

MATERIALS AND METHODS

Animals

All studies were performed under protocols approved by the Columbia University Institutional Animal Care and Use Committee in accordance with FDA regulations.

Fifty-six 300 g male Sprague Dawley rats (Charles River Laboratories, Wilmington, MA) were acclimated to a climate and light cycle controlled environment for no less than 24 hours prior to investigations. Rats were fed standard laboratory rodent chow and tap water ad libitum.

Laser

A continuous-wave Helium-Neon (HeNe) laser (Hughes Aircraft Co., Carlsbad, CA) with a 4 mWatt power output fiberoptically delivered coherent, polarized, monochromatic light (wavelength, 632.8 nm). Laser power measurements were made daily with a power meter (Newport Research, Model 815, Fountain Valley, CA)

Surgical Procedures

Excisional model. Rats were anesthetized with ketamine (33 mg/kg) and xylazine (5 mg/kg) and dorsal fur was shaved. Full-thickness skin excisions (1.5 cm in diameter) were made in the dorsal midline under aseptic conditions. To create the defect, the skin was grasped and lifted with a ring clamp and then excised with a scalpel blade. All wounds were left undressed and all animals were housed in individual cages specially designed to prevent bedding from entering the wound.

Incisional model. Rats were anesthetized with ketamine (33 mg/kg) and xylazine (5 mg/kg) and dorsal fur was shaved. A single 2 cm longitudinal full thickness skin incision was made in the dorsal midline. The incision was closed with surgical skin staples.

Treatment

For excisional wounds, a constant spot of 1.77 cm^2 (1.5 cm diameter) was irradiated for varying lengths of time to achieve the desired fluence. At an average power output of 4 mWatts and a constant spot area of 1.77 cm^2 exposure for 7 minutes and 23 seconds delivered a total dose of 1 J/cm^2; 14 minutes and 46 seconds delivered 2 J/cm^2, and 29 minutes and 32 seconds delivered 4 J/cm^2.

For incisional wounds, a constant spot of 3.142 cm^2 (2 cm diameter) was irradiated for 26 minutes and 11 seconds to deliver a total dose of 2 J/cm^2.

Experiments

Study 1A. Two 1.5 cm diameter full-thickness skin excisions were made in the dorsal midline of eight rats under aseptic conditions. The wounds were separated by a distance of 5 cm on the rostral-caudal axis. On each rat, either the

rostral or the caudal wound was designated for treatment; the remaining wound was used as a control. With the rats anesthetized, the treatment wounds on each rat received a daily HeNe dose of 1 J/cm^2. The second wound on each animal served as its own control and received no treatment. All eschars were debrided by blunt dissection daily.

Measurements of wound area were performed every other day for a period of 16 days. Two perpendicular diameters were measured using calipers. The average was then used to calculate wound area based on the assumption of a circular shape ($A = \pi r^2$). Measurements were not blinded.

Study 1B. Twenty-four rats were randomized into three groups of eight each. A single 1.5 cm diameter full-thickness skin excision was made in the dorsal midline of each rat. Group 1 served as control and received no treatment. With the rats anesthetized, group 2 wounds received a daily HeNe dose of 2 J/cm^2. Under identical conditions, group 3 wounds received a daily HeNe dose of 4 J/cm^2. All animals, including controls, were anesthetized and all eschars were debrided by blunt dissection daily. Measurements of wound area were blinded, but otherwise performed and calculated as in Study 1A for a period of 2 weeks. In addition, a total dose of 4 J/cm^2 was delivered to the bulbs of mercury and red dye containing thermometers.

Study 2. A single 2 cm longitudinal full thickness skin incision was made in the dorsal midline of 24 rats. The wounds were closed with surgical skin staples. Twelve rats served as the treatment group and received daily HeNe dose of 2 J/cm^2. The remaining 12 rats served as anesthesia controls. All rats were anesthetized daily.

Seven rats (4 control and 3 treatment) were sacrificed on postoperative day 7. Skin staples were removed prior to tensile strength determination. Three skin strips measuring ~1 × 3 cm were taken perpendicular to each incision. No more than 5 minutes after harvest, cross-sectional areas were determined by calipers and strips were mounted on a T10 tensometer (Monsanto, Akron, OH), with a crosshead speed of 10 mm/min, for determination of peak breaking strength. Individual tensile strength determinations were averaged to arrive at a mean value for each animal. The remaining 16 rats (8 control and 8 treatment) were sacrificed on postoperative day 14. Skin was harvested and data was collected as above. Caliper measurements and tensile strength determinations were blinded in Study 2.

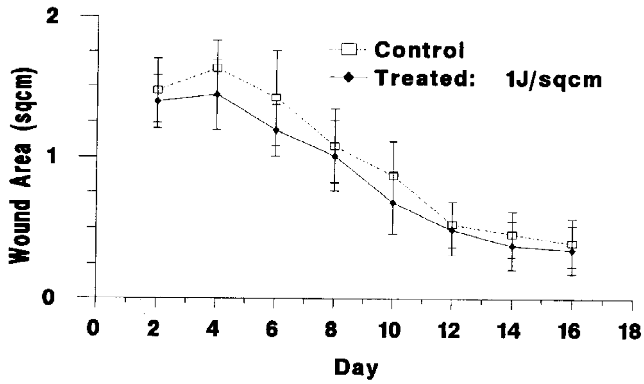

Fig. 1. Calculated wound area is plotted against time in days for control and treated excisional wounds. Each point represents the average area of eight wounds, four caudal and four rostral. ANOVA demonstrated no significant difference at any time point between groups.

Statistics

Wound areas and tensile ANOVA and strength measurements were analyzed using Student's t-test. A power analysis was performed for each study.

RESULTS

Study 1A

This pilot study was performed to establish an excisional skin contracture model. Calculated wound area is displayed graphically in Figure 1 for each time point. Each data point represents the average area of eight wounds, four caudal and four rostral. Analysis by Student's t-test demonstrated no difference between groups ($P > 0.1$ at all time points). This study had a power of > 75% to find a 50% difference with an alpha of 0.05 on postoperative days 2–10. The power of the study was small at the last two time points.

Study 1B

No differences were found among the treated and control groups at any time point. Figure 2 shows wound area plotted versus time. Each data point represents the average area of eight individual wounds. The control and treated groups are essentially indistinguishable. This study had a power of > 80% to find a 40% difference with an alpha of 0.05 at all time points tested.

Exposing the mercury and red dye containing thermometers to a fluence of 4 J/cm^2 produced no change in temperature.

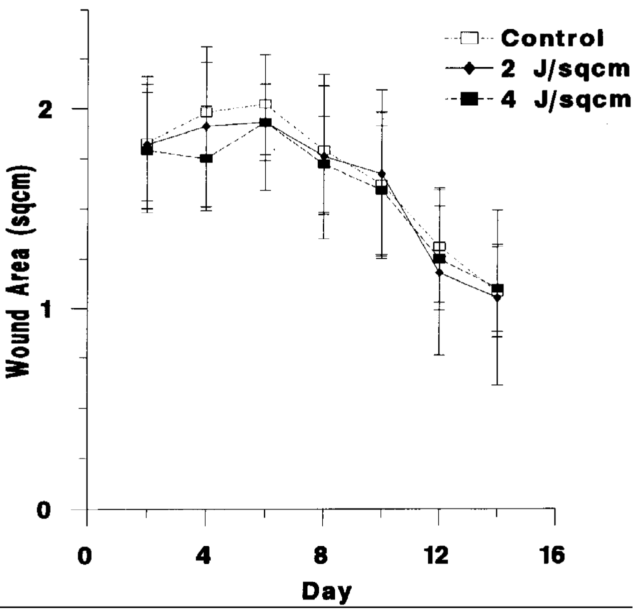

Fig. 2. Calculated wound area is plotted against time in days for control and treated excisional wounds. Each point represents the average area of eight individual wounds. ANOVA demonstrated no significant difference at any time point among groups.

Study 2

Average tensile strengths and standard deviations for treatment and control groups are shown in Table 1. Tensile strength data at postoperative days 7 and 14 showed no differences between groups ($P > 0.8$ by student's t-test). One of the 7-day rats died due to systemic injection of anesthesia and resulting overdose.

The power of this study was 77% to show a 50% difference with an alpha of 0.05 on postoperative day 14.

DISCUSSION

The literature provides considerable conflicting data on low-energy laser biostimulation of wound healing. In vitro experiments support the hypothesis that low energy laser irradiation may accelerate wound healing. Demonstration of increased collagen synthesis in vitro [16] suggests that tensile strength of incisional wounds might be increased in treated wounds. Studies that show that HeNe irradiation produces a massive transformation of fibroblasts into myofibroblasts [12] suggest the possibility of an increased rate of wound contracture in response to laser treatment. Although these cellular and biochemical events are well documented, the application of this knowledge to wound healing acceleration has been frustrated by conflicting reports. Lyons [11] succeeded in demonstrating accelerated healing in an incisional model and Kana [5] found significant differences in an excisional model. Mester [9] has produced positive results in both models, whereas several authors have reported only negative results [3, 13–15]. Strikingly, both Braverman [1] and Surinchak [4] independently reported increased tensile strength in an incisional model, but failed to produce increased contraction rates in an excisional model. Using both an excisional and an incisional model similar to those described by earlier reports, our laboratory was unable to show any beneficial effects of HeNe laser stimulation.

Study 1A had three shortcomings that necessitated modification. (1) The mechanism of HeNe biostimulation is unknown and may involve systemic factors. With control and experimental wounds on the same animal, it is not possible completely to isolate control wounds from potential systemic biochemical products of laser treatment. (2) We observed that rostral wounds contracted faster than caudal wounds irrespective of treatment. (3) Although we recognized that caliper measurements allow for subjectivity, the study was not blinded. Thus a smaller effect may have been observed due to a confounding systemic factor, or a larger effect may have been reported due to unblinded investigator bias. To address these concerns we altered the model for Study 1B. Although we overcame the initial difficulties, we still were unable to show differences between control and treated wounds.

Power analysis demonstrated that Study 1B had > 80% likelihood of finding a 40% difference at all time points tested. Our study may not have been able to detect < 40% difference; however, an effect that produces < 40% difference may not be of clinical use.

In Study 2, we attempted to demonstrate differences in an incisional model. Daily treatments of 2 J/cm^2 on skin incisions produced no beneficial effect in wound strength as compared to controls on postoperative day 7 and 14 as assessed by tensile strengths of incisional wounds. Power analysis demonstrates that Study 2 had a 77% likelihood of detecting a 50% difference on postoperative day 14. As argued for Study 1B, although a small difference may have been missed, small benefits may have limited clinical utility.

The many variable dosage and delivery pa-

TABLE 1. Tensile Strength Average (kg/cm² ± standard deviation)*

	Control	Treatment
Postoperative day 7	1.6409 ± 0.5871 (n = 3)	1.7420 ± 0.3912 (n = 4)
Postoperative day 14	6.0151 ± 1.6535 (n = 8)	5.8671 ± 1.1953 (n = 8)

*Number of animals per group is indicated.

rameters may in part explain the lack of reproducibility of results among laboratories. Several different types of lasers, including ruby, argon, and HeNe, have been used, each requiring a unique set of parameters to be effective. Fluence (J/cm^2) is often reported, but length of exposure and laser power output can be paired in an infinite number of ways to produce a single fluence value. For a given fluence, it is unclear what length of exposure coupled with power output may be optimal for treatment. In addition, researchers must control for thermal effects to ensure that observations are not produced by a heating phenomenon.

The excisional model, which has proven to be the most difficult to reproduce, has several other variables that may confound the data. Laser light penetration of tissue and eschar debridement are also concerns. Although a He-Ne laser transluminates a human finger, many researchers debride eschars daily before treatments, as we did. In fact, Surinchak [4] reports that debrided wounds contract faster than those left intact, irrespective of laser treatment. Moreover, anesthesia produces systemic effects that may retard or augment the wound healing process. If the effects of anesthesia are profound enough, they may obscure or overwhelm the effects of laser treatment. If the effects of low energy HeNe laser irradiation are too subtle to be demonstrated in a reproducible way in the laboratory, this treatment modality may be of limited clinical importance.

In summary, we attempted to establish both an excisional and an incisional model of low-energy laser biostimulation. At fluences of 1, 2, and 4 J/cm^2, the rate of wound contracture among treatment groups was essentially identical to that of controls. Also, at two postoperative time points, tensile strengths of incisional wounds were not different between treatment (2 J/cm^2) and control groups. Power analysis demonstrates that whereas small differences may have been missed, differences of 40–50% would have been detected. We suggest that low energy helium-neon laser irradiation has not been proven to have a significant impact on wound healing.

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